

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently Amended) A method for preparing a conjugate vaccine in commercial volumes, the method comprising:

reacting a polysaccharide with an oxidizing agent, whereby a solution of an aldehyde-activated polysaccharide is obtained;

reacting a protein with hydrazine dichloride at an acidic pH, whereby a solution of a hydrazine-activated protein is obtained;

purifying said solution of hydrazine-activated protein under conditions standardized to process at least five liters of solution;

reacting the aldehyde-activated polysaccharide with the hydrazine-activated protein at a pH of from ~~about~~ 5 to ~~about~~ 7 in the presence of sodium cyanoborohydride, whereby a conjugate is obtained;

neutralizing unreacted aldehyde groups with adipic acid dihydrazide; and

purifying the resulting solution under conditions standardized to process a volume of at least two liters,

whereby a conjugate vaccine capable of stimulating an immune response is obtained in commercial volumes.

2. (Original) The method according to claim 1, wherein the oxidizing agent comprises NaIO₄.

3. (Original) The method according to claim 1, wherein the solution of the aldehyde-activated polysaccharide is buffer exchanged with a HEPES buffer.

4. (Currently Amended) The method according to claim 1, wherein the solution of the aldehyde-activated polysaccharide is buffer exchanged to a pH of from ~~about~~ 7 to ~~about~~ 8.

5. (Original) The method according to claim 1, wherein the solution of the hydrazine-activated protein is buffer exchanged with a Na₂CO₃ buffer.

6. (Currently Amended) The method according to claim 1, wherein the solution of the hydrazine-activated protein is buffer exchanged to a pH of from ~~about~~ 10.0 to ~~about~~ 11.0.

7. (Currently Amended) The method according to claim 6, wherein a pH of the solution of the hydrazine-activated protein is raised to from ~~about~~ 7.0 to ~~about~~ 11 before the solution of the hydrazine-activated protein is buffer exchanged to a pH of from ~~about~~ 10.0 to ~~about~~ 11.0.

8. (Currently Amended) The method according to claim 1, wherein the aldehyde-activated polysaccharide is reacted with the hydrazine-activated protein at a ratio ~~of~~ from ~~about~~ 1:1.6 to ~~about~~ 1:5.

9. (Previously Presented) The method according to claim 1, wherein said purifying the resulting solution comprises the step of diafiltrating the conjugate vaccine, whereby substantially all unreacted compounds and unconjugated polysaccharides are removed, yielding a purified conjugate vaccine.

10. (Original) The method according to claim 9, further comprising the step of concentrating the purified conjugate vaccine by tangential flow ultrafiltration, yielding a concentrated purified conjugate vaccine.

11. (Original) The method according to claim 10, further comprising the step of adding saccharose as a stabilizer to the concentrated purified conjugate vaccine, yielding a stabilized conjugate vaccine.

12. (Original) The method according to claim 10, further comprising the step of freeze drying the concentrated purified conjugate vaccine, yielding a dried conjugate vaccine.

13. (Original) The method according to claim 1, wherein the polysaccharide is selected from the group consisting of Meningococcal polysaccharides, Pneumococcus polysaccharides, Hemophilus influenzae type b polysaccharide, Vi polysaccharide of Salmonella typhi, and group B Streptococcus polysaccharides.

14. (Original) The method according to claim 1, wherein the protein is selected from the group consisting of tetanus toxoid, diphtheria toxoid, CRM₁₉₇, and meningococcal protein.

15. (Currently Amended) A method for preparing a conjugate vaccine in commercial volumes, the method comprising:

reacting a polysaccharide with an oxidizing agent, whereby a solution of an aldehyde-activated polysaccharide is obtained;

buffer exchanging the solution of the aldehyde-activated polysaccharide to a pH ~~of~~ from about 7 to ~~about~~ 8;

reacting a protein with hydrazine dichloride at an acidic pH, whereby a solution of a hydrazine-activated protein is obtained;

raising a pH of the solution of the hydrazine-activated protein to ~~from about~~ 7.0 to ~~about~~ 11 and thereafter buffer exchanging the solution of the hydrazine-activated protein to a pH ~~of~~ from ~~about~~ 10.0 to ~~about~~ 11.0;

purifying said solution of hydrazine-activated protein under conditions standardized to process at least five liters of solution;

Application No. 10/566,898
Amendment Dated 4/7/2010
Reply to Office Action of 10/7/2009

reacting the aldehyde-activated polysaccharide with the hydrazine-activated protein at a pH of from about 5 to about 7 in the presence of sodium cyanoborohydride, whereby a conjugate is obtained;

neutralizing unreacted aldehyde groups with adipic acid dihydrazide; and purifying the resulting solution under conditions standardized to process a volume of at least two liters,

whereby a conjugate vaccine capable of stimulating an immune response is obtained in commercial volumes.

16. (Currently Amended) The method according to claim 15, wherein the aldehyde-activated polysaccharide is reacted with the hydrazine-activated protein at a ratio of from about 1:1.6 to about 1:5.

17. (Previously Presented) The method according to claim 15, wherein said purifying the resulting solution comprises the step of diafiltrating the conjugate vaccine, whereby substantially all unreacted compounds and unconjugated polysaccharides are removed, yielding a purified conjugate vaccine.

18. (Previously Presented) The method according to claim 17, further comprising the step of concentrating the purified conjugate vaccine by tangential flow ultrafiltration, yielding a concentrated purified conjugate vaccine.

19. (Previously Presented) The method according to claim 18, further comprising the step of adding saccharose as a stabilizer to the concentrated purified conjugate vaccine, yielding a stabilized conjugate vaccine.

20. (Previously Presented) The method according to claim 15, wherein the polysaccharide is selected from the group consisting of Meningococcal polysaccharides, Pneumococcus polysaccharides, *Hemophilus influenzae* type b polysaccharide, Vi polysaccharide of *Salmonella typhi*, and group B *Streptococcus* polysaccharides, and wherein the protein is selected from the group consisting of tetanus toxoid, diphtheria toxoid, CRM₁₉₇, and meningococcal protein.